#937

THE COUNCIL FOR TOBACCO RESEARCH–U.S.A., INC.

110 EAST 59TH STREET
NEW YORK, N. Y. 10022
(212) 421-8885

Application for Research Grant
(Use extra pages as needed)

Date 8/15/73

1. Principal Investigator (give title and degrees):

Marvin A. Sackner, M.D.

2. Institution & address:

Mount Sinai Medical Center
4300 Alton Road
Miami Beach, Florida 33140

3. Department(s) where research will be done or collaboration provided:

Division of Pulmonary Diseases
Department of Internal Medicine

4. Short title of study:
Effects Of Smoking On Mucociliary Clearance In Man

5. Proposed starting date: January 1, 1974

6. Estimated time to complete: 3 Years

7. Brief description of specific research aims:

1. To determine the acute effects of the smoke of tobacco products on tracheal mucous velocity in non-smokers and smokers.

2. To establish whether or not filters or additives to cigarettes such as menthol have a quantitative difference on tracheal mucous velocity during both short and long term exposure.

3. To perform tests of small airway obstruction in young smokers and to correlate the results of these tests with measurements of tracheal mucous velocity.

4. To test whether pharmacologic agents and environmental factors such as humidity and temperature modify the effects of smoking.

5. To collect and study the composition of mucus in anesthetized dogs by the methods of Proctor and his associates and to study the effects of smoking on changes in composition.

6. To develop lavage methods using the bronchofiberscope for collection of mucus and alveolar macrophages first in animals and later in man, after these methods are standardized, to study the effects of smoking on the various parameters.

7. To test the acute effects of smoking on viscosity of mucus and to measure the ciliary activity of ciliated epithelium by an in-vitro technique.

8. Brief statement of working hypothesis:

Although there is a great deal of evidence from the Surgeon General's Office that the smoking of tobacco products is associated with an increased incidence of pulmonary diseases, and that this information has been conveyed to the public, it has not cut down on the consumption of tobacco products. Many experienced physicians in the field of Pulmonary Disease recognize that it is impossible to prohibit or legislate bans on the smoking of cigarettes, owing to the fact that people simply enjoy this habit. However, it is important to distinguish characteristics among the various tobacco products that make them safer to the lungs. The mucociliary system constitutes an effective protective mechanism against inhaled particulate matter and its failure is often implicated in bronchopulmonary disease. Indeed, throughout the NHLI Task Force Report on Research in Pulmonary Diseases, it was emphasized that the study of pulmonary defense mechanisms, specifically, mucociliary clearance, was of prime importance if diminution in mortality and morbidity in lung diseases were to be achieved. Published reports on the effects of acute and chronic cigarette smoking on mucociliary clearance have been conflicting. Both increased and decreased clearance have been observed and undoubtedly some of the controversy has been related to the previous qualitative methodology for estimation of mucous clearance. Also, standardization of smoking in animals has been difficult and this may also have contributed to the difficulty in arriving at definitive conclusions. Recently we developed a cine-

(Continued on page 2A)

9. Details of experimental design and procedures (append extra pages as necessary)

1. A cine bronchofiberscopic technique for estimation of tracheal mucous velocity will be employed in paid volunteers and patients. If particles are placed on the mucosal surface, ciliary activity will carry them upward with the mucous blanket toward the larynx. Their movement can be filmed through a bronchofiberscope whose tip is located within the lumen of the airway of interest. Since the distal lens of this is wide angle and has an extremely small aperture, the image on the fibers is in satisfactory focus for object distances from 5 to 50 mm. The particles appear larger as they approach the lens of the bronchofiberscope. By standardizing the particle size and the film projection factor and knowing the filming speed, it is possible to compute their velocities from their projected image size.

   In the absence of reference distances in the trachea, the distance of a Teflon disc from the distal lens of the bronchofiberscope must be determined from its image size in the projected film. Therefore, we employed a model of the trachea in order to establish the relation between the projected image size of the disc and its distance from the lens of the bronchofiberscope. The model trachea was mounted on an optical bench and a millimeter rule fixed within it. Teflon discs were placed at various distances between 20 and 50 mm away from the distal lens of the bronchofiberscope. The bronchofiberscope was centered within the model trachea so that the distal lens lay 8 mm radially from the millimeter rule. A 16-mm Beaulieu "Endo" motion picture camera was coupled to the bronchofiberscope and the illumination by the xenon light source (Olympus model CLX) set to "maximal brightness." Kodak Ektachrome (Daylight ASA 160) was used for documentation. The processed films were reviewed on a film viewer-reader (V/R 100-C/M Traid Corp., Glendale, Calif.). This rear projection viewer with a fixed magnification permits measurements of positions on the screen from mechanical counters coupled to manually controlled x and y cross hairs. The film frame may be read from a mechanical counter.

   The cinefilms are viewed on the same film viewer-reader used for analysis of the films taken in the model trachea. Each particle whose velocity is to be computed is viewed at a minimum of three positions (frames) in the course of its filmed path. At each of these positions, the major axis of the elliptical particle image is located and measured by moving the cross hair intersection to the boundaries of this image on the axis. The disc diameter which forms the major axis of the image is always perpendicular to the line from the tip of the bronchofiberscope to the particle. For this reason, the major axis of the image depends only on the distance of the disc, not on the angle from which it is viewed. This increases the insensitivity of our method to decentering of the bronchofiberscope. Choice of frames

9. Brief statement of working hypothesis (Continuation)

bronchofiberscopic method that can be employed in humans and animals to obtain serial measurements of tracheal mucous velocity over prolonged time intervals. A logical extension of our research activities is to study the effects of smoking on tracheal mucous velocity, to develop methods of collecting and analyzing mucus, to integrate histologic examination of ciliated epithelium and the alveolar macrophage in order to achieve a better understanding of the effects of smoking on these host defense mechanisms.

9. Details of experimental design and procedures (Continuation)

from which image size is to be recorded must also be made in such a way as to isolate mucous motion from motion of the tracheal wall due to respiration and heartbeat. This is accomplished by choosing a fixed point on the viewing screen and taking measurements only from frames in which some tracheal landmark coincides with this point. Dark fixed spots due to broken optical fibers are readily available screen points and tracheal rings are convenient tracheal landmarks. If no dark spot falls on a tracheal ring, a contour is drawn with a grease pencil on the viewing screen to coincide with the tracheal ring in frames from which data are taken. All measurements for velocity computation are made at resting lung volume (FRC) position.

To compute a mean tracheal mucous velocity, 10-12 particles are measured in each run. We trace lines on the viewing screen to divide the trachea into four quadrants and try to measure image size of three to four particles in each quadrant.

a. Acute effects of cigarette smoking. Ten light or nonsmokers and ten heavy smokers will be given cigarettes standardized for tobacco and nicotine content and will be asked to smoke them in a standardized fashion. Comparisons will be made on the acute effects of smoking different cigarettes on tracheal mucous velocity on separate days. Observations will be carried out over a 30 minute period and should mucous velocity be depressed, administration of catecholamines will be given in an effort to speed it up. These subjects will also be asked to abstain from smoking one to two weeks and the experiments repeated.

b. Subjects who are known to have small airway obstruction (see screening tests for estimation of small airway disease) versus cigarette smokers matched for pack years but who do not have evidence of small airway disease, will be selected. Tracheal mucous velocity will be measured in both groups to ascertain whether impairment of this parameter is an earlier sign of pulmonary dysfunction than smaller airway disease. Attempts to modify mucous clearance will be accomplished by administration of catecholemines and other pharmacologic agents.

c. The effects of preconditioning with air of low humidity or high humidity and high or low temperature will be studied to obtain an understanding of the interrelationship of environmental factors in the response to smoking. The subjects will be placed within a environmental chamber presently in our laboratory prior to smoking a cigarette and then tracheal mucous velocity will be measured.

d. The effects of standardized cigarettes supplied by the Tobacco Industry will will be employed to derive quantitative differences on tracheal mucous velocity among various brands of tobacco products.

2. Tests of small airway obstruction to correlate with measurements of tracheal mucous velocity. There has been a great deal of emphasis recently on the detection of small airway disease by means of various proposed tests. These include measure of closing volume, analysis of the flow volume curve, analysis of single breath nitrogen test, measurement of the frequency dependence and measurement of frequency dependence of lung compliance. The latter is thought to be the most sensitive technique and also is the standard for discrimination among normal and diseased subjects in the presence of otherwise normal pulmonary function. However, it is a invasive test involving the swallowing of an esophageal balloon and therefore is unsatisfactory for widespread screening. We have recently confirmed a mathematical link between frequency dependence of lung compliance and distribution of ventilation, the latter determined by the nitrogen washout technique. Assuming a two compartment system with equal compliances in making corrections for Pendelluft and common dead space mixing effects,

9. Details of experimental design and procedures (Continuation)

the ratio of dynamic to static lung compliance for any respiratory frequency can be calculated from the compartmental analysis of the nitrogen washout at a single respiratory frequency. Using these equations, a good correlation was found between calculated and measured C_{L_D}/C_{L_S} in a mechanical lung model, in dogs with artificially induced bronchial obstruction, and in young smokers or young non-smokers following carbachol inhalation. Finally, a two compartment nitrogen washout was demonstrated in 10 healthy smokers at one or two respiratory frequencies whereas all 10 normal controls showed a single exponential curve. These findings indicate that the non-invasive nitrogen washout test is capable of predicting C_{L_D}/C_{L_S} and at the same time gives a direct measure of gas distribution. Further, it appears to be a highly sensitive method for the detection of "small airway disease".

As mentioned above, we will attempt to screen smokers with similar smoking histories for evidence of small airway disease and those with no evidence for this and ascertain whether mucous clearance parallels these tests, whether the suppression of mucous clearance is found only in those subjects with the small airway disease or whether it is not found at all. These patients will then be challenged with cigarette smoke to ascertain what happens to tracheal mucous velocity.

3. Collection and Composition Of Mucus In Anesthetized Dogs or Unanesthetized Sheep. We have previously demonstrated that tracheal mucous velocity can easily be measured in anesthetized dogs. However, it is also possible to study unanesthetized sheep. We do not have facilities for housing sheep within our Animal Laboratory and we first plan to make these measurements in anesthetized dogs and later should they prove of interest in conscious sheep in an animal facility that will have to be rented outside the hospital. We intend to make measurements of the respiratory mucus after the method described by Proctor, Aharonson, Reasor and Bucklan: (Bull. Physio-Path. Resp. 9: 351-357, 1973). These authors placed a soft, plastic coated, glass fiber mesh into the upper trachea to collect mucus and an absorbent cotton wad just beneath the vocal cords. They found that the total tracheobronchial mucous flow seemed to be about 1 to 3 grams an hour. They collected the mucus from the mesh and the cotton and then washed the material from these collecting devices to study the composition of mucus. We plan to estimate the total amount of mucus as did Proctor and associates and also to measure the viscosity, total solids, nitrogenous components, electrolytes, and protein. We plan to study the acute effects of smoking and correlate it with tracheal mucous flow. Once the acute studies are completed, we then plan, some time after the initial granting period to study the chronic effects of cigarette smoking on these variables as to whether any adverse effects can be protected by the addition of additives to the smoke. For example, it has been shown by Dalhamn and Rylander, (Am. Rev. of Resp. Dis. 103: 855-857, 1971) that oxolamine citrate and Phenyl vinlyoxadiazole and Phenyl methyl-oxadiazole when added to cigarette smoke prevent a depression of ciliary activity.

4. Lavage technique for alveolar macrophage using the bronchofiberscope. We are presently developing a technique using a silastic rubber balloon at the end of the bronchofiberscope to obtain alveolar macrophages by subsgemental lavage from different portions of the lungs. We intend to first develop this technique in anesthetized animals and later to use it in patients and normal volunteers. We shall correlate tracheal mucous velocity on the one hand and alveolar macrophage recovery and activity in response to the smoke of various tobacco products. By employing subsegmental lavage, different portions of the lungs can be sampled during a given study as a function of time. Harris, Swenson and Johnson (J. Clin. Invest. 49: 2086, 1970) have previously measured phagocytic activity and glucose

9. Details of experimental design and procedures (Continuation)

utilization in human alveolar macrophages in smokers and nonsmokers. The macrophages were obtained by bronchopulmonary lavage and the studies were carried out in vitro in the absence of smoke. We plan to extend these studies by obtaining repeated subsegmental lavages as a function of time.

5. Estimation of viscosity of bronchial secretions. Although viscosity of sputum has been measured by many investigators over the past several years, results have been disappointing. This is because expectorated sputum is a nonhomogeneous material and therefore different samplings give widely varying values. This may also be true of bronchial secretions but no good data are available on this fluid owing to the prior difficulty of measuring the viscosity of a small sample. Recently, a new clinical viscometer has been designed which appears to circumvent these difficulties (Bleeker and Hoeksema: Ann. Otol. 82: 248, 1973). This device requires only .015 ml of specimen. We hope to obtain this quantity of mucus first from anesthetized dogs by developing a capillary meshwork device for sampling within the tracheobronchial tree. Later on, the mucus will be sampled for change in viscosity after acute exposure to different tobacco products.

6. In vitro determination of ciliary activity. Measurements of mucociliary clearance, as in our cine-bronchofiberscopic technique, give an overall picture of transport of mucus which is dependent on its composition, viscosity and amount, as well as on the integrity of ciliary activity. It is not possible to monitor ciliary activity in vivo but with exfoliated bronchial epithelium mounted in a nutrient wet preparation, preliminary microscopic studies indicate that ciliary activity can be measured using a visual auditory feedback technique (Bleeker, personal communication). We believe that a stroboscopic technique may be an improvement of this method for measurement of ciliary activity. We plan to develop this technique in animals and then apply it in humans smoking various tobacco products.

10. Space and facilities available (when elsewhere than item 2 indicates, state location):

On or about October, 1973 the Division of Pulmonary Diseases will be moved into a new area in the Blum Research Building, consisting of 8,000 sq. ft. Of this space, approximately 4,500 sq. ft. are devoted to laboratories for both clinical and research work. The clinical pulmonary laboratory is equipped with 1) two automatic pulmonary function devices, 2) three slowly responding helium analyzers and one rapidly responding helium analyzer, 3) three nitrogen gas analyzers, 4) two carbon monoxide infrared analyzers, 5) Pulmonet with closed circuit helium setup, 6) Collins body plethysmograph, 7) Ergometer, 8) DR-8 Electronics for Medicine oscilloscope with recorder and PR-7 recorder, 9) setup for rebreathing diffusing capacity, 10) four desk calculators, 11) one rapidly responding and two slowly responding oxygen analyzers, 12) two blood gas electrode machines, 13) one Capnograph, 14) one simulated breathing device, 15) Wolff 4 channel tape recorder. The research laboratory is equipped with 1) DR-12 Electronics for Medicine oscilloscope recorder, 2) Grass 78 - 13 channel recorder, 3) Phillips 7 channel analog tape recorder, 4) 12 channel Electronics for Medicine oscilloscope recorder, 5) horizontal body plethysmograph for combined cardiac and pulmonary studies, 6) vertical environmental chamber-body plethysmograph, 7) nitrous oxide infrared gas analyzer, 8) bag and box spirometers, 9) apparatus with associated bybrid computer for pulmonary and chest wall mechanics and for estimation of closing volumes, 11) Perkin Elmer mass spectrometer, 12) five Olympus bronchofiberscopes, two light sources for taking of cine film, and two Beleau motion picture cameras. A biochemistry support laboratory is housed in this new facility. It is equipped for radioactive isotope waste disposal.

Computer support is from two PDP-12 computers. One PDP-12 computer has 12 K core, 800,000 word disc, hard wired floating point package and a Versitek line printer. The other PDP-12 has 12 K core and a card reader. Several software packages have been developed for pulmonary function testing and for research data processing.

(Continued 3A)

11. Additional facilities required:

None

12. Biographical sketches of investigator(s) and other professional personnel (append):

13. Publications: (five most recent and pertinent of investigator(s); append list, and provide reprints if available).

(See page 3B and 3C)

10. Space and facilities available (Continuation)

On the hospital grounds is an animal research laboratory with approximately 500 sq. ft. of space. Equipment includes 1) DR-12 oscilloscope recorder, 2) horizontal body plethysmograph, 3) image intensifier and x-ray equipment with cine, 4) nitrous oxide analyzer, 5) CO_2 analyzer, 6) TMC 7 channel tape recorder, 7) Gas Chromatograph, 9) LINC 8 digital computer. Adjacent to the animal laboratory is a trailer containing offices and a rear view motion picture projector with paper tape output for preparation of data for computer processing of mucous velocity experiments.

13. Five most recent and pertinent publications:

Marvin A. Sackner, M.D.

1. Avery, W.G. and Sackner, M.A.: A rapid measurement of functional residual capacity in the paralyzed dog. J. Appl. Physiol. 33: 515-518, 1972.

2. Sackner, M.A., Wanner, A. and Landa, J.: Applications of bronchofiberscopy Chest, 62: Suppl. 2, 70S - 78S, 1972.

3. Michaelson, E.D., Sackner, M.A. and Johnson, R.L., Jr.: Vertical distributions of pulmonary diffusing capacity and capillary blood flow in man. J. Clin. Invest. 52: 359-369, 1973.

4. Sackner, M.A., Rosen, J.J. and Wanner, A.: Estimation of tracheal mucous velocity by bronchofiberscopy. J. Appl. Physiol., 34: 495-499, 1973.

5. Sackner, M.A., Rosen, M.J. and Wanner, A.: Effects of oxygen breathing and endotracheal intubation on tracheal mucous velocity of anesthetized dogs. Bull Physiol-path. Resp. 9: 403-415, 1973.

Jose F. Landa, M.D.

1. Landa, J., Avery, W.G. and Sackner, M.A.: Some physiologic observations in smoke inhalation. Chest, 61: 62-64, 1972.

2. Amikam, B., Landa, J., West, J. and Sackner, M.A.: Bronchofiberscopic observations of the tracheobronchial tree during endotracheal intubation. Am. Rev. Resp. Dis., 105: 747-755, 1972.

3. Sackner, M.A., Wanner, A. and Landa, J.: Applications of bronchofiberscopy. Chest, 62: Suppl. 2, 70S - 78S, 1972.

4. Sackner, M.A. and Landa, J.: Bronchofiberscopy: To intubate or not to intubate! Chest, 63: 302, 1973.

Adam Wanner, M.D.

1. Wanner, A., Zighelboim, A. and Sackner, M.D.: Nasopharyngeal airway: a facilitated access to the trachea. Ann. Int. Med., 75: 593-595, 1971.

2. Wanner, A., Amikam, B. and Sacknner, M.A.: A technic for bedside bronchofiberscopy. Chest, 61: 287-288, 1972.

3. Sackner, M.A., Wanner, A. and Landa, J.F.: Applications of bronchofiberscopy. Chest, 62: 705-785, 1972.

4. Sackner, M.A., Rosen, M.J. and Wanner, A.: Estimation of tracheal mucous velocity by bronchofiberscopy. J. Appl. Physiol. 34: 495-499, 1973.

5. Wanner, A. and Sackner, M.A.: Transvenous phrenic nerve stimulation in anesthetized dogs. J. Appl. Physiol., 34: 489-494, 1972.

13. Five most recent and pertinent publications (Continuation)

Edward D. Michaelson, M.D.

1. Walsh, R.E., Michaelson, E.D., et al.: Upper airway obstruction in obese patients with sleep disturbance and somnolence. Ann. Int. Medicine, 76: 185-192, 1972.

2. Michaelson, E.D., Sackner, M.A., and Johnson, R.L., Jr.: Vertical distributions of pulmonary diffusing capacity and capillary blood flow in man. J. Clin. Invest. 52: 359-369, 1973.

3. Crossley, R.J., Leverett, S.D., Jr., Shubrooks, S.J., Jr., Michaelson, E.D., and Burton, R.R.: Human physiologic responses to high, sustained $+G_Z$ acceleration. Preprints of Scientific Program, Aerospace Medical Association, May 7-10, 1973.

4. Shubrooks, S.J., Jr., Leverett, S.D., Jr., Crossley, R.J., Michaelson, E.D., and Burton, R.R.: Human physiologic responses to high, sustained $+G_Z$ with positive pressure breathing. Preprints of Scientific Program, Aerospace Medical Association, May 7-10, 1973.

5. Michaelson, E.D., O'Byrne, B.: Effects of varying inspired oxygen concentrations (FIO_2) on pulmonary mechanics. Preprints of Scientific Program, Aerospace Medical Association, May 7-10, 1973.

14. First year budget:

A. Salaries (give names or state "to be recruited")	% time	Amount
Professional (give % time of investigator(s) even if no salary requested)		
Marvin A. Sackner, M.D.	15	0
Adam Wanner, M.D.	10	3,600
Jose Landa, M.D.	33	12,000
Edward Michaelson, M.D.	17	6,000
Biochemist (to be named)	100	18,500
Technical		
Research Technician (to be named)	100	9,500
Pulmonary Technician	30	0
Sub-Total for A		49,600
B. Consumable supplies (by major categories)		
Cine Film and Processing		4,500
Glassware, disposables		3,000
Animals		4,000
Chemicals, reagents		2,500
Sub-Total for B		14,000
C. Other expenses (itemize)		
Fees for Volunteers (for bronchofiberscopic studies)		8,000
Travel		500
Publication Costs		1,000
Sub-Total for C		9,500
Running Total of A + B + C		73,100
D. Permanent equipment (itemize)		
Viscometer for microanalysis of mucus (for measuring mucous viscosity)		3,000
Series H20 Advanced Phase Microscope Model H20 TG-P-8 with photographic accessories (for measurement of ciliary beating of bronchial epithelial cells)		4,400
Sub-Total for D		7,400
E. Indirect costs (15% of A+B+C)		10,965
Total request		91,465

15. Estimated future requirements:

	Salaries	Consumable Suppl.	Other Expenses	Permanent Equip.	Indirect Costs	Total
Year 2	52,328	14,000	9,500	0	11,374	87,202
Year 3	55,206	15,000	9,500	0	11,956	91,662

16. Other sources of financial support:
List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Volumes And Compliance Of Pulmonary Circulation	NIH-HL 10622	200,825	9/1/66-8/31/75
Effects Of Oxygen On Mucous Clearance Rates	NIH-71-2205	101,715	7/1/71-7/1/74
Detection And Prevention Of Regional Pulmonary Atelectasis, Edema, And Hypoperfusion During Acceleration	Aerospace Med. Div. F 41609-72-C-004	98,832	10/14/71-10/14/73
Pulmonary Training Grant	1 TO1 NL5980-01	200,000	7/1/73-7/1/78

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Host Defense Mechanisms Of The Lung	NHLI	Application Incomplete	5/1/74-5/1/77
Detection and Prevention Of Regional Pulmonary Atelectasis, Edema, And Hypoperfusion During Acceleration	Aerospace Med. Div.	49,900	10/14/73-10/14/74

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Principal investigator

Typed Name Marvin A. Sackner, M.D.

Signature Marvin A. Sackner Date 8/15/73

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Responsible officer of institution

Typed Name Samuel Gertner

Title Executive Vice President

Signature [signature] Date 8/17/73

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